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(74) Agent: GREENFIELD, Michael, S.; McDonnell
Boehnen Hulbert & Berghoff, 300 South Wacker Drive,
Chicago, IL 60606 (US).

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(71) Applicants (for all designated States except US): AVEN-
TIS PASTEUR [US/US]; 1 Discovery Drive, Swiftwa-
ter, PA 18370-00187 (US). UAB RESEARCH FOUNDA-
TION [US/US]; Suite 1120G, 701 South 20th Street, Birm-
ingham, AL 35294-0111 (US).

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(72) Inventors; and

(75) Inventors/Applicants (for US only): NABORS, Gary, S.
[US/US]; 210 Park Avenue, Stroudsburg, PA 18360 (US).
BRILES, David [US/US]; 760 Linwood Road, Birming-
ham, AL 35222 (US).



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(54) Title: ANTIBODY-BASED TREATMENT FOR *STREPTOCOCCUS PNEUMONIAE* INFECTION

(57) Abstract: The present invention comprises a method of treating a mammal infected with *Streptococcus pneumoniae*, which methods comprises administering to the mammal a therapeutically effective amount of one or more PspA antibodies. Preferably the mammal is a human.

ANTIBODY-BASED TREATMENT FOR *STREPTOCOCCUS PNEUMONIAE* INFECTION

BACKGROUND OF THE INVENTION

This application claims the benefit of U.S. Provisional Application No. 60/139,524,
5 filed June 16, 1999.

Field of the Invention

The present invention relates to the therapeutic use of PspA antibodies for the treatment of *Streptococcus pneumoniae* infection in mammals, particularly in a human.

Summary of the Related Art

10 *Streptococcus pneumoniae* is an important cause of otitis media, meningitis, bacteremia and pneumonia, and a leading cause of fatal infections in the elderly and persons with underlying medical conditions, such as pulmonary disease, liver disease, alcoholism, sickle cell, cerebrospinal fluid leaks, acquired immune deficiency syndrome (AIDS), and patients undergoing immunosuppressive therapy. It is also a leading cause of morbidity in
15 young children. Pneumococcal infections (i.e. infections caused by *Streptococcus pneumoniae*) cause approximately 40,000 deaths in the U.S. yearly. The most severe pneumococcal infections involve invasive meningitis and bacteremia infections, of which there are 3,000 and 50,000 cases annually, respectively.

Despite the use of antibiotics and vaccines, the prevalence of pneumococcal infections
20 has declined little over the last twenty-five years; the case-fatality rate for bacteremia is reported to be 15-20% in the general population, 30-40% in the elderly, and 36% in inner-city African Americans. Less severe forms of pneumococcal disease are pneumonia, of which there are 500,000 cases annually in the U.S., and otitis media in children, of which there are an estimated 7,000,000 cases annually in the U.S. caused by pneumococci. Strains of drug-
25 resistant *S. pneumoniae* are becoming ever more common in the U.S. and worldwide. In some areas, as many as 30% of pneumococcal isolates are resistant to penicillin. The increase in antimicrobial resistant pneumococci further emphasizes the need for either preventing pneumococcal infections or developing a therapeutic regimen to cure those already infected.

Historically, antisera prepared in animals to the pneumococcal capsule was used
30 therapeutically to treat bacteremic/septic pneumonia. Since virtually all fatal pneumonia is due to bacteremic/septic pneumonia, the procedure was of an significant medical value especially in the pre-antibiotic era. Treatment with antisera in combination with good nursing care served to decrease the death rate among patients infected with *Streptococcus pneumoniae*. At that time antibodies were prepared in rabbits or horses to whole killed

bacteria of each capsular type. Different capsular polysaccharide types differentially facilitate virulence in humans and other species. Historically, the capsular type of the infecting patient was determined by the Quellung reaction or a mouse protection test and then an antiserum to the appropriate capsular polysaccharide type was given to the patient parenterally. These treatments were published prior to the wide spread use of antibiotics (White, B. 1938. The Biology of Pneumococcus. The Commonwealth Fund, New York; Heffron, R. 1939. Pneumonia. The Commonwealth Fund, New York). Using these methods, it was necessary for the attending physician to know the polysaccharide type of the pneumococcus prior to administering a specific antiserum, since antisera to most specific capsule types are not cross-reactive.

Protection mediated by anti-capsular polysaccharide antibody responses are capsular type specific. Different polysaccharide types differentially facilitate virulence in humans and other species. In total, there are at least 90 different capsular polysaccharide types of *S. pneumoniae* that cause disease in man (Heinrichsen, J. Six newly recognized types of *Streptococcus pneumoniae* J. Clin. Micro. 33: 2759-62 (1995)). The 23 most common capsular types represent about 90% of the pneumococcal disease in adults. These 23 polysaccharide types have been used in a licensed pneumococcal vaccine since 1983 (D.S. Fedson, M. Musher, Vaccines, S.A. Plotkin and J.E.A. Montimer, eds., 1994, pp. 517-564). The efficacy of the vaccine has been controversial, and at times the justification for the recommended use of the vaccine questioned. It has been speculated that the efficacy of this vaccine is negatively affected by having to combine 23 different antigens. Having a large number of antigens combined in a single formulation may negatively affect the antibody responses to individual types within this mixture because of antigenic competition. The efficacy is also affected by the fact that the 23 serotypes do not encompass all serological types associated with human infections and carriage. Consequently, a therapeutic antibody-based treatment for infections with *S. pneumoniae* which uses antibodies to capsular polysaccharide is problematic in that it is not possible to cover all the strains that are infectious in man.

Therefore, there exists a need for a therapeutic treatment for *S. pneumoniae* that is simple, efficacious for all infectious *S. pneumoniae* strains, and is not limited by antibiotic resistance.

McDaniel *et al.* described in 1984 an immunogenic, species-common protein from *S. pneumoniae*, designated pneumococcal surface protein A (McDaniel LS, G Scott, JF

Kearney, DE Briles. Monoclonal antibodies against protease sensitive pneumococcal antigens can protect mice from fatal infection with *Streptococcus pneumoniae*. J. Exp. Med. 160:386-397, 1984). The term "PspA" was first used in a title of a paper published in 1987 (McDaniel LS, J Yother, L Vijayakumar, M McGarry, WR Guild, DE Briles. Use of insertional inactivation to facilitate studies of biological properties of pneumococcal surface protein A (PspA). J. Exp. Med. 165:381-394, 1987); see also U.S. patent nos. 5,728,387 and 5,679,768.

PspA from *S. pneumoniae* contains choline-binding repeats that are responsible for the attachment of the PspA to the surface of the pneumococcus. PspA molecules interfere with complement activation, slow clearance of pneumococci from the blood of infected mice and elicit protection against pneumococcal sepsis, otitis media, and nasal carriage. Although variable, antibody responses raised to PspA are cross-reactive to heterologous PspA proteins and immunization with one PspA can protect against pneumococci that contain a heterologous PspA and capsule type (Crain *et al.* Pneumococcal surface protein A (PspA) is serologically highly variable and is expressed by all clinically important capsular serotypes of *Streptococcus pneumoniae* Infect. Immun. 58: 3293-99 (1990)). Such cross-protective immunity has been shown using truncated PspA proteins of various lengths (McDaniel *et al.*, Location of protection-eliciting epitopes on PspA of *Streptococcus pneumoniae* between amino acid residues 192 and 260; Microb. Pathogen 17: 323-37 (1994)). A protective region spanning amino acids 192-270 has been used as the basis for organizing PspA proteins into three families further subdivided into six clades (Briles *et al.*, PspA and PspC: their potential for use as pneumococcal vaccines; Microb. Drug Resist. 3: 401-08 (1997); see also U.S. 5,955,089). PspA family 1 is composed of clades 1 and 2, family 2 is composed of clades 3-5 and family 3 includes only clade 6. Clade 6 is most dissimilar from all other clades. Over 97% of the PspA molecules typed to date have been found to be members of families 1 and 2. Furthermore, PspA shows cross-reactivity with the related *S. pneumoniae* antigen, PspC.

SUMMARY OF THE INVENTION

The present invention comprises a method of treating a mammal infected with *Streptococcus pneumoniae*, which method comprises administering to the mammal a therapeutically effective amount of one or more PspA antibodies. Preferably the mammal is a human.

In another aspect, the present invention comprises the use of PspA antibodies in the manufacture of a medicament for the treatment of *Streptococcus pneumoniae* infection in a mammal (preferably a human).

Due to the cross-reactive nature of anti-PspA antibodies, the method of the invention is independent of the specific polysaccharide type of the infectious *S. pneumoniae* strain. Therefore, unlike the prior art, the present invention obviates the need both to type the *S. pneumoniae* as well as the need to use tens of different antigens from various *S. pneumoniae* strains to produce antibodies that cover the spectrum of different infectious *S. pneumoniae* strains. The method of the present invention provides a less complex approach to treatment and leads to reduced delay in patient treatment.

Until now it was unknown whether *S. pneumoniae* expressed PspA during infection and, therefore, it was uncertain whether passive anti-PspA antibody treatment would be effective against an existing infection; without expression, treatment with anti-PspA antibodies clearly would be ineffective. We have discovered that *S. pneumoniae* does in fact express PspA during infection. Furthermore, it was uncertain whether sufficient amount of antibody could be delivered to the multiple foci of bacterial infection to effectively treat it. As demonstrated below, we have found that despite the uncertainties inherent in the prior art, passive anti-PspA antibody treatment is effective against *S. pneumoniae* infection.

The foregoing merely summarizes certain aspects of the invention and is not meant to limit the invention in any manner. All patents and other publications recited in this specification are hereby incorporated by reference in their entirety.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention comprises the use of PspA antibody as a therapeutic against *S. pneumoniae* infection in a mammal (preferably human). The antibodies for use in the present invention can be generated by immunizing humans or animals with one or more PspA molecules. Immunoglobulin for therapeutic use could be purified from plasma using the standard Cohn-Oncley method (Cohn EJ, Strong LE, Hughes WI Jr, *et al.* Preparation and properties of serum and plasma proteins. IV: A system for the separation into fractions of protein and lipoprotein components of biological tissues and fluids. J Am Chem Soc 1946;68:459-75. Oncley JL, Melin M, Richert DA, Cameron JW, Gross PM Jr. The separation of the antibodies isoagglutinins, prothrombin, plasmonogen and beta-lipoprotein into sub-fractions of human plasma. J Am Chem Soc 1949;71:541-50) or a modification of this method.

As described in the Background of the Invention, there are numerous strains of *S. pneumoniae*. In a preferred embodiment of the present invention, more than one PspA molecule from different strains of *S. pneumoniae* is utilized in the production of PspA antibodies. In a more preferred embodiment of the present invention, PspA proteins from
5 three to five different *S. pneumoniae* strains (preferably from different clades) is used to generate PspA antibodies. In another preferred embodiment of the present invention, PspA proteins from clades 2, 3, and 4 are used to generate PspA antibodies. In another preferred embodiment of the present invention, PspA proteins from clades 1, 2, 3, 4 and 6 are used to generate PspA antibodies. In a particularly preferred embodiment of the present invention
10 PspA proteins containing truncated forms of PspA proteins from clades 1, 2, 3, 4 and 6 are used to generate PspA antibodies. As noted, antibodies raised against PspA (or one or more of its fragments) from one or more different strains and/or clades are administered to a subject (preferably human) infected with *S. pneumoniae*.

In an alternate embodiment of the present invention, the antibody preparation is made
15 from the serum of non-immunized individuals with naturally high titers of anti-PspA antibody.

The term "PspA" refers to pneumococcal surface protein A and fragments thereof including but not limited to PspA/Rx1. The term PspA includes but is not limited to mutant proteins of naturally occurring PspA that retain at least one epitope of the naturally occurring
20 PspA, such as PspA/Rx1MI. A number of U.S. patents disclose various PspA fragments. E.g., U.S. patent nos. 5,980,909, 5,965,141, 5,871,943, 5,856,170, and 5,804,193.

The term PspA/Rx1 refers to a protein that is N-terminal region (aa1-314) of the naturally occurring PspA polypeptide of Rx1 (GenBank Accession No. M74122 and WO 92/14488). The *S. pneumoniae* strain Rx1 has been deposited with the American Type
25 Culture Collection, 12301 Parklawn Drive, Rockville, Md. 20852 under accession no. 55834.

The term PspA/Rx1MI refers to a protein that is the N-terminal region (aa1-314) of the naturally occurring PspA polypeptide of Rx1 in which amino acid 96 has been changed from a methionine to an isoleucine.

The terms "PspA antibody" and "anti-PspA antibody" refer to any antibody or group
30 of antibodies to PspA, PspA/Rx1, or PspA/Rx1MI, as defined above. PspA antibodies include but are not limited to polyclonal antibodies to PspA, monoclonal antibodies to PspA, monospecific polyclonal antibodies to PspA, and mixtures thereof.

The phrase "therapeutically effective amount" as used herein means an amount of PspA antibody sufficient to eliminate (preferably) an *S. pneumoniae* infection in the treated mammal, or halt or reduce the rate of growth of bacterial burden in the infected mammal.

The PspA antibodies for use in the present invention, used as a therapeutic agent, can be delivered in the form of an antiserum, an IgG fraction from an antiserum, or as a pooled IgG + IgM fraction from an antiserum. Additionally, the PspA antibodies of the present invention could be purified from antiserum by purification techniques, many of which are well known in the art, such as immunoaffinity columns.

In another embodiment of the present invention, administration of PspA antibody is combined with administration of antibiotics or other antibodies to *S. pneumoniae* antigens. Such therapeutic use of the PspA antibodies is anticipated to improve the recovery time of the patient, or his/her likelihood of survival. It is envisioned that therapy with the invention would be unlikely to inhibit the activity of antibiotics when both agents are co-administered.

The present invention has a distinct advantage over other conceivable similar substances, such as a polyvalent anti-pneumococcal polysaccharide serum, because of the high level of cross-reactivity in the anti-PspA humoral response. Due to the cross-reactive nature of anti-PspA antibodies, the specific polysaccharide or PspA type of the infectious *S. pneumoniae* strain would not limit the usefulness of the invention. Therefore, unlike the prior art, in the present invention there is no need to type the *S. pneumoniae*, and, thus, methods of the present invention are less complex and lead to reduced delay in patient treatment.

The amount of PspA antibody to be administered to a mammal infected with *Streptococcus pneumoniae* and the regime of administration can be determined in accordance with standard techniques well known to those of ordinary skill in the pharmaceutical and veterinary arts taking into consideration such factors as the particular antigen, the adjuvant (if present), the age, sex, weight, species and condition of the particular animal or patient, and the route of administration. In the present invention, the amount of specific antibody administered to provide an efficacious therapeutic dose for treatment of *S. pneumoniae* can be from between about 0.02 mg to about 5 mg per kg body weight. In a preferred composition and method of the present invention the dosage is between about 0.1 mg to 3 mg per kg of body weight. The optimal dosage will depend upon the post-infection time of administration. For example, an efficacious dosage will require less antibody if the post-infection time elapsed is less since there is less time for the bacteria to proliferate. In like manner an

efficacious dosage will depend on the bacterial load at the time of diagnosis. Multiple injections administered over a period of days could be considered for therapeutic usage.

5 Examples of compositions of the invention include liquid preparations for orifice, *e.g.*, oral, nasal, anal, vaginal, peroral, intragastric, mucosal (*e.g.*, perlingual, alveolar, gingival, olfactory or respiratory mucosa) etc., administration such as suspensions, syrups or elixirs; and, preparations for parenteral, subcutaneous, intradermal, intramuscular, intraperitoneal or intravenous administration (*e.g.*, injectable administration), such as sterile suspensions or emulsions. Intravenous and parenteral administration are preferred. Such compositions may be in admixture with a suitable carrier, diluent, or excipient such as sterile water,
10 physiological saline, glucose or the like. The compositions can also be lyophilized. The compositions can contain auxiliary substances such as wetting or emulsifying agents, pH buffering agents, gelling or viscosity enhancing additives, preservatives, flavoring agents, colors, and the like, depending upon the route of administration and the preparation desired. Standard texts, such as "REMINGTON'S PHARMACEUTICAL SCIENCE", 17th edition,
15 1985, incorporated herein by reference, may be consulted to prepare suitable preparations, without undue experimentation.

Compositions of the invention are conveniently provided as liquid preparations, *e.g.*, isotonic aqueous solutions, suspensions, emulsions or viscous compositions that may be buffered to a selected pH. If digestive tract absorption is preferred, compositions of the
20 invention can be in the "solid" form of pills, tablets, capsules, caplets and the like, including "solid" preparations which are time-released or which have a liquid filling, *e.g.*, gelatin covered liquid, whereby the gelatin is dissolved in the stomach for delivery to the gut. If nasal or respiratory (mucosal) administration is desired, compositions may be in a form and dispensed by a squeeze spray dispenser, pump dispenser or aerosol dispenser. Aerosols are
25 usually under pressure by means of a hydrocarbon. Pump dispensers can preferably dispense a metered dose or a dose having a particular particle size.

Liquid preparations are normally easier to prepare than gels, other viscous compositions, and solid compositions. Additionally, liquid compositions are somewhat more convenient to administer, especially by injection or orally, to animals, children, particularly
30 small children, and others who may have difficulty swallowing a pill, tablet, capsule or the like, or in multi-dose situations. Viscous compositions, on the other hand, can be formulated within the appropriate viscosity range to provide longer contact periods with mucosa, such as the lining of the stomach or nasal mucosa.

Obviously, the choice of suitable carriers and other additives will depend on the exact route of administration and the nature of the particular dosage form, *e.g.*, liquid dosage form (*e.g.*, whether the composition is to be formulated into a solution, a suspension, gel or another liquid form), or solid dosage form (*e.g.*, whether the composition is to be formulated into a pill, tablet, capsule, caplet, time release form or liquid-filled form).

Solutions, suspensions and gels, normally contain a major amount of water (preferably purified water) in addition to the active ingredient. Minor amounts of other ingredients such as pH adjusters (*e.g.*, a base such as NaOH), emulsifiers or dispersing agents, buffering agents, preservatives, wetting agents, jelling agents, (*e.g.*, methylcellulose), colors and/or flavors may also be present. The compositions can be isotonic, *i.e.*, it can have the same osmotic pressure as blood and lacrimal fluid.

The desired isotonicity of the compositions of this invention may be accomplished using sodium chloride, or other pharmaceutically acceptable agents such as dextrose, boric acid, sodium tartrate, propylene glycol or other inorganic or organic solutes. Sodium chloride is preferred particularly for buffers containing sodium ions.

Viscosity of the compositions may be maintained at the selected level using a pharmaceutically acceptable thickening agent. Methylcellulose is preferred because it is readily and economically available and is easy to work with. Other suitable thickening agents include, for example, xanthan gum, carboxymethyl cellulose, hydroxypropyl cellulose, carbomer, and the like. The preferred concentration of the thickener will depend upon the agent selected. The important point is to use an amount that will achieve the selected viscosity. Viscous compositions are normally prepared from solutions by the addition of such thickening agents.

A pharmaceutically acceptable preservative can be employed to increase the shelf-life of the compositions. Benzyl alcohol may be suitable, although a variety of preservatives including, for example, parabens, thimerosal, chlorobutanol, or benzalkonium chloride may also be employed. A suitable concentration of the preservative will be from 0.02% to 2% based on the total weight although there may be appreciable variation depending upon the agent selected.

Those skilled in the art will recognize that the components of the compositions must be selected to be chemically inert with respect to the PspA antibodies.

EXAMPLES

Example 1

Production of PspA/Rx1MI

The production of PspA/Rx1 is described in Nabors *et al.*, Vaccine 18:1743-1754 (2000). See, also, WO 99/14333 for the production of PspA/RX1MI. In general, cells transformed with a plasmid containing the DNA encoding the first 314 amino acids of PspA from Rx1 were grown to mid-exponential phase and induced with 0.5 mM IPTG (Sigma, Inc.). Cells were harvested approximately 2 hours after induction by centrifugation. Cell pellets were resuspended in a PBS-lysozyme lysis buffer (0.5 mg/ml lysozyme in PBS). The cells were lysed by freeze thaw and the lysate was centrifuged.

The supernatant was decanted and adjusted to 70% ammonium sulfate (J.T. Baker) to precipitate proteins. This solution was centrifuged and the supernatant was discarded. The pellet was resuspended in 30% ammonium sulfate and centrifuged. The supernatant was extensively dialyzed with a buffer consisting of 100 mM sodium acetate, 10 mM NaCl, pH 4.5. The dialysate was centrifuged and the supernatant was applied to a Q Sepharose Fast Flow (Pharmacia Biotech) column. The column flow through and wash were collected and dialyzed against 50 mM Tris, 2 mM EDTA, 10 mM NaCl, pH 7.5. The dialysate was centrifuged and the supernatant was applied to a Q Sepharose Fast Flow column. The column was washed with 50 mM Tris, 2 mM EDTA, 100 mM NaCl, pH 7.5, then Rx1MI was eluted with 50 mM Tris, 2 mM EDTA, 200 mM NaCl, pH 7.5. This eluate was analyzed by SDS-PAGE; proteins were visualized by Coomassie Blue staining and PspA products were identified by Western immunoblot analysis with monoclonal anti-PspA.

The process described in Nabors *et al.* (Vaccine 18), WO 99/14333, and in this example can be used (and routinely modified as needed) to express and purify essentially any PspA protein.

Example 2

Production of PspA Antibody

420 BALB/c female mice (21 groups of 20 mice) age 7-8 weeks were interperitoneally (i.p.) injected with 0.5 ml of 25 mM Tris buffered saline containing 520 µg/ml Alhydrogel and the concentration of PspA/Rx1MI listed below:

Table 1

Group	Mass of PspA/Rx1MI (µg)	Injection Schedule
1	0	Day 0

Group	Mass of PspA/Rx1MI (μ g)	Injection Schedule
2	250	Day 0
3	62.5	Day 0
4	15.625	Day 0
5	3.906	Day 0
6	0.977	Day 0
7	0.244	Day 0
8	0.061	Day 0
9	0.0153	Day 0
10	0.00382	Day 0
11	0	Day 0 + 21
12	250	Day 0 + 21
13	62.5	Day 0 + 21
14	15.625	Day 0 + 21
15	3.906	Day 0 + 21
16	0.977	Day 0 + 21
17	0.244	Day 0 + 21
18	0.061	Day 0 + 21
19	0.0153	Day 0 + 21
20	0.00382	Day 0 + 21
21	0	Day 0 + 21

After 35 days all mice were bled via the brachial artery. 100 μ l of blood from each mouse in groups 12-17 were pooled and sterile filtered (0.45 μ m). The filtrate was separated into 24 - 0.5 ml aliquots each containing 433.7 μ g/ml of PspA antibody as measured by ELISA.

5

Example 3

Therapeutic Administration of Pooled PspA Antibody: Strain WU2

30 BALB/c female mice (3 groups of 10 mice) age 7-8 weeks were infected with 300 CFU of *S. pneumoniae* WU2 (capsule type 3, PspA clade 2). Group 1 mice were bled six hours after infection with WU2 to assess blood CFU levels, and were then injected i.p. with 1.302 μ g PspA antibody derived from Example 2. Group 2 mice were bled twelve hours after infection with WU2 and then injected i.p. with 1.302 μ g PspA antibody derived from Example 2. Group 3 mice were bled twelve hours after infection with WU2 and then injected

i.p. with Ringer's lactate solution. All mice were then bled 24 h after the first bleed (30 h for group 1 and 36 h for groups 2 and 3). A second dose of PspA antibody (1.302 μ g) was administered i.p. 48 hours after the first administration, 54 and 60 h for groups 1 and 2, respectively. The results of the study are listed below:

5

Table 2

Mouse	Dose	CFU in 2 nd Bleed	CFU in 54/60 h bleed	Death (days)
Group 1-1	Anti-Rx1MI @ 6h		< 1.7	>28
Group 1-2	Anti-Rx1MI @ 6h		< 1.7	>28
Group 1-3	Anti-Rx1MI @ 6h		Contaminated	>28
Group 1-4	Anti-Rx1MI @ 6h		< 1.7	>28
Group 1-5	Anti-Rx1MI @ 6h		< 1.7	>28
Group 1-6	Anti-Rx1MI @ 6h		Contaminated	>28
Group 1-7	Anti-Rx1MI @ 6h		< 1.7	>28
Group 1-8	Anti-Rx1MI @ 6h		Contaminated	>28
Group 1-9	Anti-Rx1MI @ 6h		< 1.7	>28
Group 1-10	Anti-Rx1MI @ 6h		< 1.7	>28
Group 2-1	Anti-Rx1MI @ 12h		< 1.7	>28
Group 2-2	Anti-Rx1MI @ 12h		< 1.7	>28
Group 2-3	Anti-Rx1MI @ 12h		< 1.7	>28
Group 2-4	Anti-Rx1MI @ 12h		< 1.7	>28
Group 2-5	Anti-Rx1MI @ 12h		< 1.7	>28
Group 2-6	Anti-Rx1MI @ 12h		< 1.7	>28
Group 2-7	Anti-Rx1MI @ 12h		3.8	8
Group 2-8	Anti-Rx1MI @ 12h		< 1.7	>28
Group 2-9	Anti-Rx1MI @ 12h		< 1.7	>28
Group 2-10	Anti-Rx1MI @ 12h		< 1.7	>28
Group 3-1	Ringer's @ 12h	> 6.7	Dead	2
Group 3-2	Ringer's @ 12h	> 6.7	Dead	2
Group 3-3	Ringer's @ 12h	> 6.7	Dead	2
Group 3-4	Ringer's @ 12h	5.5	Dead	2
Group 3-5	Ringer's @ 12h	6.1	Dead	2
Group 3-6	Ringer's @ 12h	6.3	Dead	2

Mouse	Dose	CFU in 2 nd Bleed	CFU in 54/60 h bleed	Death (days)
Group 3-7	Ringer's @ 12h	3.0	4.2	6
Group 3-8	Ringer's @ 12h	> 6.7	Dead	2
Group 3-9	Ringer's @ 12h	5.2	Cont.	3
Group 3-10	Ringer's @ 12h	Dead	Dead	1

Example 4

Therapeutic Administration of Pooled PspA Antibody: Capsule Types 3, 4, 5, and 6B

80 mice (CBA/N mice from Jackson Labs) were divided into four groups of 20 each.

The mice in each group were infected i.v. with *S. pneumoniae* as follows:

- 5 Group 1: 2.7 x 10⁴ CFU/mouse of DBL5 (capsule type 5/PspA clade 2);
- Group 2: 840 CFU/mouse of EP5668 (capsule type 4/PspA clade 4);
- Group 3: 116 CFU/mouse of BG7322 (capsule type 6B/PspA clade 2); and
- Group 4: ~1000 CFU/mouse of 3JYA4 (capsule type 3/PspA clade 3).

10 Within each group, the mice were further divided into four subgroups of five mice each as follows:

1. Mice receiving passive antibody before infection and no further treatment;
2. Mice receiving passive NMS (normal mouse serum, 1/10 dilution) before infection and no further treatment;
3. Mice infected and treated at 6, 12, and 30 hours with antibody; and
- 15 4. Mice infected and treated at 6, 12, and 30 hours with NMS.

All mice were bled for CFU counts at 6 hours after infection. The mice to receive antibody or NMS post-infection were treated with 100 µl of 1/10 dilution of serum in Ringer's lactate. This process was repeated at 12 and 30 hours post-infection. The results are summarized in Table 4:

20

Table 3

Group	Sub-group	Treatment	Days to Death	Median Days to Death	Alive: Dead	Geometric Mean of Log CFU @ 6 hours	Geometric Mean of Log CFU @ 12 hours	P vs NMS for 12 hr CFU
1	A1-A5	Pass. Ab + DBL5	5x 1	1	0:5	2.12	4.25	0.0005
	A6-10	Tx. Ab + DBL5	5x 1	1	0:5	3.78	6.49	0.3659
	B1-B5	Pass. NMS + DBL5	2x 0, 3x 1	1	0:5	3.34	6.93	0.0005
	B6-10	Tx. NMS + DBL5	0, 4x 1	1	0:5	3.41	6.81	0.3659
2	C1-C5	Pass. Ab + EF5668	3x 3, 8,9	3	0:5	<1.49	1.62	0.3467
	C6-10	Tx. Ab + EF5668	1,2,2,3,13	2	0:5	<1.49	1.49	0.3854
	D1-D5	Pass. NMS+ EF5668	2,2,3,10,11	3	0:5	<1.49	<1.49	0.3467
	D6-10	Tx. NMS + EF5668	2,10,12,12,>20	12	1:4	<1.49	1.58	0.3854
3	E1-E5	Pass. Ab + BG7322	5x >5	>20	5:0	1.83	<1.49	<0.0001
	E6-10	Tx. Ab + BG7322	2x5,8,8,>20	8	1:4	2.82	3.91	0.0002
	F1-F5	Pass. NMS+ BG7322	1, 4x 2	2	0:5	3.21	4.91	<0.0001
	F6-10	Tx. NMS + BG7322	4x 1, 2	1	0:5	3.06	5.14	0.0002
4	G1-15	Pass. Ab + 3JYA4	2x 0, 3x 1	1	0:5	<1.49	N/A	
	G6-10	Tx. Ab + 3JYA4	5x 1	1	0:5	<1.49	N/A	
	H1-H5	Pass. NMS + 3JYA4	0, 3x 1, 2	1	0:5	<1.49	N/A	
	H6-10	Tx. NMS + 3JYA4	4x 1, >20	1	1:4	1.60	N/A	

The data for Group 3 demonstrate that treatment of *S. pneumoniae* infected mice significantly increased the life span of infected mice and reduced the bacterial burden relative to mice administered NMS. Mice in groups 1, 2, and 4 treated with PspA antibody did not manifest

increased life span or reduced bacterial burden. In each of groups 1, 2, and 4, however, not even passive immunization before infection (when bacterial load is at a minimum) was effective, indicating that groups 1, 2, and 4 are examples in which the amount or specificity of anti-PspA delivered to the infected animals was insufficient for the bacterial burden delivered and, thus, did not constitute a therapeutically effective dose of PspA antibody.

Example 5

Therapeutic Administration of Pooled PspA Antibody.

In this example, 42 CBA/N mice were divided into 6 groups of 7 animals:

1. Those infected with A66.1 and treated with PspA antibody at 6 hours and 54 hours post-infection
2. Those infected with A66.1 and treated with PspA antibody at 12 hours and 60 hours post-infection
3. Those infected with BG7322 and treated with PspA antibody at 24 hours and 72 hours post-infection
4. Those infected with BG7322 and treated with PspA antibody at 48 hours and 96 hours post-infection
5. Those infected with A66.1 and treated with Ringer's lactate
6. Those infected with BG7322 and treated with Ringer's lactate

All mice were bled for CFU counts prior to each treatment. All treated mice received 0.1 ml of a 1/100 dilution of the immune serum described in Example 2. The data are given in Tables 5 and 6.

Table 4

1. A66.1 @ 6 hours			Cage A		Mice received 240 CFU at time 0	
Ms #	Log CFU at 6 hr	Log CFU at 30 hr	Log CFU at 54 hr	Log CFU at 144 hr	Date of Death	Days to Death
1-1	3.0	<1.7	<1.7	<1.7	alive	>16
1-2	3.0	<1.7	<1.7	<1.7	alive	>16
1-3	3.5	<1.7	<1.7	<1.7	alive	>16
1-4	3.4	4.2	3.5	<1.7	alive	>16
1-5	3.0	3.4	3.9	dead	6-14-99 @ 9a	6
1-6	<1.7	<1.7	<1.7	<1.7	alive	>16
1-7	3.2	2.8	3.8	dead	6-12-99 @ 5p	4

2. A66.1 @ 12 hours Cage B Mice received 240 CFU at time 0						
Ms #	Log CFU at 12 hr	Log CFU at 36 hr	Log CFU at 60 hr	Log CFU at 144 hr	Date of Death	Days to Death
2-1	<1.7	<1.7	1.9	ND	alive	>16
2-2	5.3	4.5	4.7	4.4	6/20/99	10
2-3	4.8	4.6	dead	dead	6-10-99 @ 9a	2
2-4	2.9	<1.7	<1.7	3.8	alive	>16
2-5	4.2	3.4	6.2	dead	6-11-99 @10p	3
2-6	4.8	4.1	6.1	dead	6-11-99 @ 9a	3
2-7	4.8	4.3	4.5	5.0	6-18-99 @ 3p	8
3. BG7322 @ 24 hours Cage C Mice received 2,216 CFU at time 0						
Ms #	Log CFU at 24 hr	Log CFU at 48 hr	Log CFU at 72 hr	Log CFU at 144 hr	Date of Death	Days to Death
3-1	4.7	4.7	5.6	4.6	6-15-99 @10a	7
3-2	5.1	5.1	6.3	dead	6-12-99 @ 5p	4
3-3	5.6	3.9	6.7	dead	6-12-99 @ 5p	4
3-4	5.1	4.9	>6.7	dead	6-11-99 @10p	3
3-5	5.6	4.1	6.1	dead	6-14-99 @ 9a	6
3-6	5.5	3.1	3.7	ND	6-17-99 @10a	9
3-7	5.8	4.0	3.9	4.1	6-17-99 @10a	9
4. BG7322 @ 48 hours Cage D Mice received 2,216 CFU at time 0						
Ms #	Log CFU at 48 hr	Log CFU at 72 hr	Log CFU at 96 hr	Log CFU at 144 hr	Date of Death	Days to Death
4-1	5.2	ND	dead	dead	6-12-99 @ 5p	4
4-2	5.2	ND	>6.7	dead	6-14-99 @ 9a	6
4-3	5.1	ND	4.4	4.9	6-18-99 @ 3p	10
4-4	4.8	ND	3.3	dead	6-14-99 @ 9a	6
4-5	3.8	ND	3.4	5.5	6-16-99 @ 2p	8
4-6	4.6	ND	dead	dead	6-12-99 @ 5p	4
4-7	ND	ND	5.7	6.0	6-14-99 @ 4p	6

5. A66.1 Ringers Only Cage E Mice received 240 CFU at time 0						
Ms #	Log CFU at 12 hr	Log CFU at 36 hr	Log CFU at 60 hr	Log CFU at 144 hr	Date of Death	Days to Death
5-1	4.8	dead	dead	dead	6-10-99 @ 8a	2
5-2	3.8	>6.7	dead	dead	6-9-99 @ 8p	1
5-3	1.9	>6.7	dead	dead	6-9-99 @ 9a	1
5-4	4.9	dead	dead	dead	6-9-99 @ 8p	1
5-5	4.7	dead	dead	dead	6-10-99 @ 9a	2
5-6	4.5	>6.7	dead	dead	6-9-99 @ 8p	1
5-7	4.4	dead	dead	dead	6-10-99 @ 8p	2
6. BG7322 Ringers only Cage F Mice received 2,216 CFU at time 0						
Ms #	Log CFU at 48 hr	Log CFU at 72 hr	Log CFU at 96 hr	Log CFU at 144 hr	Date of Death	Days to Death
6-1	3.8	ND	3.6	3.6	6-16-99 @ 2p	8
6-2	2.7	ND	>6.7	dead	6-14-99 @ 9a	6
6-3	3.7	ND	>6.7	dead	6-14-99 @ 9a	6
6-4	3.5	ND	>6.7	dead	6-14-99 @ 9a	6
6-5	3.6	ND	3.1	2.9	6/30/99 @ 2p	22
6-6	4.4	ND	4.9	5.3	6-18-99 @ 3p	10
6-7	3.5	ND	3.1	2.6	6-15-99 @10a	7

ND = not determined

Table 5

Gr.	Mean log CFU values at each time point													
	6h	12h	24h	30h	36h	48h	54h	60h	72h	96h	144h	Days to Death	Median day of death	P versus Ringers
1	2.96			2.40			2.51				1.6	4.6, 5x 16	>16	0.0006
2		4.06			3.44			4.17			4.4	2,3,3,8,10,>16,>16	8	0.0012
3			5.34			4.26			5.59		4.35	3,4,4,6,7,9,9	6	0.2593
4						4.78				4.72	5.47	4,4,3x 6,8,10	6	0.2086
5		4.14			6.80							4x 1, 3x 2	1	N/A
6						3.6				5.01	3.6	3x6,7,8,10, 22	7	N/A

N/A = not applicable

In this example, strain A66.1 (capsule type 3, PspA clade 2) and BG7322 (capsule type 6B, PspA clade 2) were used to establish infections prior to administration of antiserum. It was of note that significant survivorship or extension of life occurred in antibody-treated mice previously infected with strain A66.1. While it was possible to demonstrate a curative effect of the anti-PspA serum for strain A66.1 in Example 6, no benefit of treatment with specific anti-PspA antibody was observed for stain BG7322. However, as in the case of Example 5, this would be an example in which the amount or specificity of anti-PspA delivered to the infected animals was insufficient for the infectious dose administered. In this case, the infectious dose of BG7322 was 2216 CFU, almost 20-fold higher than that which was used in Example 5.

Example 6

Therapeutic effect of treatment with monoclonal antibody to PspA

Methods CBA/N mice were infected with 148 CFU of A66.1 (capsule type 3, PspA clade 2) and were subsequently treated at the indicated times below with 10 µg or 20 µg of XiR278 (IgG1 monoclonal anti-PspA described in Crain MJ, Waltman WD, Turner JS, Yother J, Talkington DF, McDaniel LS, Gray BM and Briles DE. *Pneumococcal surface protein A (PspA) is serologically highly variable and is expressed by all clinically important capsular serotypes of *Streptococcus pneumoniae*. Infect Immun 1990;58:3293-3299*). Five to eight mice per group were used. The results are give below.

Table 6

Treatment	Antibody Dose (µg)	Day of Death	Median day of death	Alive : Dead	P versus Infection only (two tailed Wilcoxon)
SET 1					
Infection only, untreated	20	5x 1	1	0 : 5	--
XiR278 at 1 h post infection	20	2, 4, 9, 5x >21	>21	5 : 3	0.0016
XiR278 at 6 h post infection	20	7x 4, >21	4	1 : 7	0.0016
SET 2					
Infection only, untreated	10	4x 1, 2x 2	1	0 : 6	--
XiR278 at 6 h post infection	10	3x 3, 10, >21	3	1 : 4	0.0043
XiR278 at 12 h post infection	10	3x 3, 9, 13	3	0 : 5	0.0043
XiR278 at 24 h post infection	10	3x 1, 2x 2	1	0 : 5	0.93 (n.s.)

n.s. = not significant

We concluded from this study that therapy with monoclonal anti-PspA antibody could cause the resolution of an established, otherwise fatal pneumococcal infection in mice. In this example, more dramatic results were observed when antibody was administered shortly (1 h) after infection, as opposed to later. However, a significant delay in the time to death was
5 observed when 20 μ g of antibody was administered at 6-12 h post-infection.

The foregoing descriptions of the various embodiments of the invention have been presented for purposes of illustration and description. They are not intended to be exhaustive or to limit the invention to the precise form disclosed, and many obvious modifications and variations by those skilled in the art are possible in light of the above teaching.

We Claim:

1. A method of treating a mammal infected with *S. pneumoniae*, the method comprising administering to the mammal a therapeutically effective amount of one or a plurality of PspA antibodies.
2. The method according to claim 1 wherein the mammal is a human.
3. The method according to claim 1 wherein one antibody is administered.
4. The method according to claim 1 wherein a plurality of antibodies raised against PspA from three to five strains of *S. pneumoniae* is administered.
5. The method according to claim 4 wherein the PspA are from a plurality of clades.
6. The method according to claim 5 wherein the PspA are from clades 2, 3, and 4.
7. The method according to claim 5 wherein the PspA are from clades 1, 2, 3, 4 and 6.
8. The method according to claim 1 wherein at least a PspA antibody raised against PspA/Rx1 is administered.
9. The method according to claim 1 wherein at least a PspA antibody raised against PspA/Rx1MI is administered.
10. The method according to claim 1 wherein the antibody or antibodies are co-administered with an antibiotic or an antibody to a *S. pneumoniae* antigen other than PspA.

INTERNATIONAL SEARCH REPORT

In tional Application No

PCT/US 00/16581

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61P31/04 A61K39/40

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, MEDLINE, EMBASE, BIOSIS, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 14333 A (PASTEUR MERIEUX CONNAUGHT ; BECKER ROBERT (US); GRAY MARY ANN (US);) 25 March 1999 (1999-03-25) page 17; claim 17 --- -/--	1-10

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

19 September 2000

Date of mailing of the international search report

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Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl.
Fax: (+31-70) 340-3016

Authorized officer

von Ballmoos, P

INTERNATIONAL SEARCH REPORT

In. .tional Application No

PCT/US 00/16581

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>MCDANIEL L S ET AL: "Comparison of the PspA sequence from Streptococcus pneumoniae EF5668 to the previously identified PspA sequence from strain Rx1 and ability of PspA from EF5668 to elicit protection against pneumococci of different capsular types." INFECTION AND IMMUNITY, (1998 OCT) 66 (10) 4748-54. , XP000918275 page 4749, left-hand column, last paragraph page 4752, left-hand column, last paragraph -right-hand column, paragraph 1</p>	1
X	<p>NAYAK A R ET AL: "A live recombinant avirulent oral Salmonella vaccine expressing pneumococcal surface protein A induces protective responses against Streptococcus pneumoniae." INFECTION AND IMMUNITY, (1998 AUG) 66 (8) 3744-51. , XP000918253 abstract page 3748, right-hand column, paragraph 2</p>	1
X	<p>LANGERMANN S ET AL: "Protective humoral response against pneumococcal infection in mice elicited by recombinant bacille Calmette-Guerin vaccines expressing pneumococcal surface protein A." JOURNAL OF EXPERIMENTAL MEDICINE, (1994 DEC 1) 180 (6) 2277-86. , XP000918247 page 2281, left-hand column, last paragraph -right-hand column, paragraph 1</p>	1
X	<p>MCDANIEL, LARRY S. ET AL: "Monoclonal antibodies against surface components of Streptococcus pneumoniae" MONOCLONAL ANTIBODIES BACT. (1986), 143-64. EDITOR(S): MACARIO, ALBERTO J. L.; CONWAY DE MACARIO, EVERLY. PUBLISHER: ACADEMIA, ORLANDO, FLA. , XP000918405 page 156</p>	1
A	<p>BRILES D E ET AL: "PspA and PspC: their potential for use as pneumococcal vaccines." MICROBIAL DRUG RESISTANCE, (1997 WINTER) 3 (4) 401-8. REF: 61 , XP000918215 cited in the application figure 2</p>	1-10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/16581

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 1-10 are directed to a method of treatment of the mammal body, the search has been carried out and based on the alleged effects of the composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 00/16581

Patent document
cited in search report

Publication
date

Patent family
member(s)

Publication
date

WO 9914333 A

25-03-1999

AU

9574598 A

05-04-1999

EP

1015591 A

05-07-2000

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